

## Ancient Environmental DNA - Workshop

*The event is co-organised by SciLifeLab, Uppsala University, Stockholm University and the Bolin Centre for Climate Research.*

**Venue:** Rockefeller lecture hall, Nobels väg 11, KI Campus Solna

### Program

- 09.00 Registration
- 09.20 Welcome words - **Laura Parducci UU, Tanja Slotte SciLifeLab/SU, Barbara Wohlfarth SU**
- 09.30 Invited lecture: **Inger G Alsos** *Plant DNA in sediments: to which degree do they reflect the flora?*
- 10.10 **Miklós Bálint** *Metabarcoding recent anthropogenic impact on lakes,*
- 10.20 **Engy Ahmed** *Ancient DNA in lake sediments: shotgun metagenomic analyses*
- 10:30 Refreshments
- 10.50 **Anushree Sanyal** *Changes in genetic structure in sediment revived *Chaetoceros* populations*
- 11.00 **Annegret Larsen** *Have beavers engineered low-order streams and floodplains for thousands of years (before humans did)?*
- 11.10 **Omneya Ahmed Osman** *Changes in the microbial communities of a deep sediment core from Ekoln Lake*
- 11.20 Invited lecture: **Mikkel Winther Pedersen** *Shotgun metagenomic analysis on ancient lake sediments*
- 12.00 Lunch
- 13.00 Invited lecture: **Gentile Francesco Ficetola** *Long-term changes of alpine landscapes revealed by lake sediment DNA*
- 13.40 **Meriam Guellil** *Needle in a Haystack: Searching for pathogens in ancient human metagenomic datasets*
- 13.50 **Nolwenn Callac** *When lipid biomarkers meet gene specific quantification: insight into a 150-year record of phytoplankton community succession controlled by hydroclimatic variability in a tropical lake?*
- 14:00 Refreshments
- 14.30 **Mikkel Winther Pedersen** *Short course on metagenomic analyses*
- 16.00 Discussion

## Abstracts

### **Plant DNA in sediments: to which degree do they represent the flora?**

Inger Greve Alsos,<sup>1</sup> Eric Coissac,<sup>2</sup> Mary Edwards,<sup>3</sup> Marie Kristine Foreid,<sup>1</sup> Ludovic Gielly,<sup>2</sup> Per Sjøgren,<sup>1</sup> Pierre Taberlet,<sup>2</sup> and Nigel Yoccoz<sup>1</sup>

<sup>1</sup>UiT – The Arctic University of Norway, Troms, Norway; <sup>2</sup>University Grenoble Alpes, LECA Grenoble, France; <sup>3</sup>University of Southampton, Southampton, UK.

Environmental DNA (eDNA) extracted from sediments has promise as a new proxy in studies of modern biodiversity and palaeobiological reconstruction. However, little is known about how well this method represents the flora. We used the g and h universal plant primers for the short and variable P6 loop region of the chloroplast trnL (UAA) intron to amplify DNA in lake sediment samples of different ages (modern, 100-200 years, and Holocene), as well as modern soil samples. We compare this with modern vegetation and other proxies (pollen and macrofossils) to evaluate the representation of different taxonomical groups, the geographical area likely to be represented, and the taphonomy of DNA. The analyses of soil samples from the Arctic showed that all species represented in the soil DNA grew within 3 m from the sampling point, most of them within 0.5 m. Lake sediments represent the flora of the catchment area, but a lower proportion of the flora were represented in the DNA record compared to soil samples. DNA and pollen of 100–200-year-old lake sediments from regions where forest was planted in the mid-twentieth century showed that both proxies discern major vegetation change at the time of plantation, with similar quantitative changes. In a lake core from the Arctic dated to 8500-1200 cal. BP, all except two genera identified as macrofossils were also identified with DNA. Furthermore, DNA identified six additional taxa and more species per sample. With one DNA extraction and one PCR per sample, we detect most common species independent of sample type or age. Increasing the number of extractions or PCR repeats increased the chances of detecting rare species. Some taxonomic groups (e.g., Cyperaceae) were consistently underestimated, whereas others (e.g., water plants) were overrepresented compared to the other proxies. We conclude that the local flora was well represented in the DNA of the sediments, and the method may have a higher and (or) complementary taxonomic resolution than analyses of pollen or macrofossils. However, further improvements are required if we want to use of this methods to monitoring of biodiversity. We suggest genome skimming as an approach to obtain higher taxonomic resolution and present preliminary results on genome reference library for Scandinavia and the Alps.

### **Metabarcoding recent anthropogenic impact on lakes**

Miklós Bálint

*Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany*

Agricultural development followed a peculiar pattern in former communist European countries during the last 150 years, with relatively clear low-impact, intensive, and again low impact phases. I am using sediment-preserved DNA to reconstruct the structure of historic eukaryote communities during this period. The study is carried out in a German lake district, with dated cores from 20 lakes. Eukaryotic communities are currently analysed with 18S metabarcoding on Illumina. Agricultural impact is directly inferred from the sediments with C/N isotope ratios, elemental composition and persistent organic pollutant analyses. The results will be informative about the role of societal change on the communities and resilience of lakes.

### **Ancient DNA in lake sediments: shotgun metagenomic analyses**

Engy Ahmed<sup>1,2</sup>, Lu Han<sup>3,4</sup>, Mikkel Winther Pedersen<sup>5</sup>, Laura Parducci<sup>4</sup>, Tanja Slotte<sup>2,6</sup>, and Barbara Wohlfarth<sup>1</sup>

<sup>1</sup>Department of Geological Sciences, Stockholm University, Sweden; <sup>2</sup>Science for Life Laboratory, Stockholm University, Sweden; <sup>3</sup>Ancient DNA Laboratory, college of Life Sciences, Jilin University, PR China; <sup>4</sup>Department of Ecology and Genetics, Uppsala University, Sweden; <sup>5</sup>Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Denmark; <sup>6</sup>Department of Ecology, Environment and Plant Sciences, Stockholm University, Sweden

Ancient DNA (aDNA) in lake sediments opens novel approaches to a better understanding of past environmental changes in aquatic and terrestrial ecosystems. The lake sediment sequences of Hässeldala and Atteköpsmosse in southern Sweden form two excellent records to investigate the wide spectrum of aquatic and terrestrial floral, faunal and microbial remains that existed between 17000 and 11000 years ago. aDNA were extracted from 26 sediment samples and shotgun metagenomic libraries were constructed in aDNA dedicated facilities at the Centre for GeoGenetics, Copenhagen. Here we present preliminary results from the currently ongoing bioinformatic processing of the shotgun metagenomic dataset. The analyzed samples cover past cold and warm time intervals and thus have the potential to provide a high-resolution reconstruction of ancient biological diversity under very different climatic scenarios.

### **Changes in genetic structure in sediment revived *Chaetoceros* populations**

Anushree Sanyal<sup>1,2</sup>, Josefine Larsson<sup>1</sup>, Mikael Lönn<sup>1</sup>, Thomas Andrén<sup>1</sup>, and Elinor Andrén<sup>1</sup>

<sup>1</sup>School of Natural Sciences, Technology and Environmental Studies, Södertörn University, Sweden;

<sup>2</sup>Department of Organismal Biology, Uppsala University, Sweden

*Chaetoceros* is a very diverse marine diatom genus found throughout the Baltic Sea stratigraphy from the early Littorina Sea until today. Lightly silicified vegetative valves of *Chaetoceros* are rarely recovered in sediments due to dissolution, but heavily silicified resting spores have been recorded in abundance in the Baltic Sea. The resting spore formation is often a response to nutrient depletion at the termination of a phytoplankton bloom and their occurrence often coincides with high productivity events. High resting spore abundance is found during the Holocene thermal maximum, the Medieval Climate Anomaly and during the last ca. 50 years and has been used as a proxy for high productivity events.

Resting stages in the sediment are of ecological importance as they can be woken up when exposed to suitable environmental conditions and are a source of genetic material for micro-evolutionary studies. Regional factors like climate, nutrient concentrations, oceanographic conditions and anthropogenic disturbances have an effect on these species. Paleoenvironmental information is lost due to the difficulty in differentiating between the resting spores of various *Chaetoceros* taxa.

We are examining the genetic diversity in *Chaetoceros* populations from sediments drilled in the Landsort Deep within and across ca. 100 years, ca. 1000-1300 and ca. 6000-7000 calendar years BP, to trace the evolutionary changes in these species. Genetic markers will be identified to determine the changes in the population genetic structure over time in *Chaetoceros* populations grown from revived fossil spores. Comparative genomics will be used to understand the evolutionary changes in the species due to environmental or anthropogenic effects and to gain insights into the mechanisms of molecular evolution.

Resting spores from all three time periods have been germinated. Nine populations from different sediment layers from the last ca. 50 years have been grown. ITS, *rbcL*, *psbA*, TBP and EFL gene regions from the nine populations were amplified. Genetic variation between populations within the same layer and different layers will be examined. Single cell DNA extraction has been performed on the older germinated spores. The results of the DNA analyses will be compared with traditional diatom stratigraphical analyses to evaluate and strengthen environmental interpretations, and to cross validate results of a paleogenomic approach.

## **Have beavers engineered low-order streams and floodplains for thousands of years (before humans did)?**

Annegret Larsen and Stuart Lane

*Institute for Earth Surface Dynamics, University of Lausanne, Switzerland*

Well incised gravel bed, meandering streams, bordered by a self-formed, fine-grained floodplain are the perceived ideal for the central European mountainous area. By analyzing ancient environmental DNA from Holocene, pre-Medieval wetland deposits in combination with dating and mapping post-Medieval floodplain sedimentation and dams, we aim to find out if the presence of beavers in and influence upon low-order central European rivers and floodplains had created an ecosystem long lost to widespread Medieval settlement expansion. Implications lie in river restoration, especially in a Swiss and European context, where current legislation increasingly forces the implementation of river restoration projects in the upcoming decades.

## **Changes in the microbial communities of a deep core sediment from Ekoln Lake**

Omneya Ahmed Osman and Stefan Bertilsson

*Department of Ecology & Genetics, Limnology, Uppsala University, Norbyv. 18D  
SE-75236, Uppsala, Sweden*

Lake sediments can reveal the diversity and shift in microbial communities over time. We collected a deep (359 cm) sediment core from Ekoln Lake, Sweden. Sediment samples were sliced at 10 cm intervals to map the historical change in deposited and immobilized microbial community subsets. The age of the sediments dates several thousand years ago. Illumina sequencing targeting 16S rRNA revealed the presence of 3 distinctive clusters of samples from high depth to the surface.

## **Shotgun metagenomic analysis on ancient lake sediments**

Mikkel Winther Pedersen

*Centre for GeoGenetics, The Natural History Museum of Denmark, Øster Voldgade 5-7, 1350  
Copenhagen K, Denmark*

Over time lakes are filled in with components from the surrounding environment creating a stacked archive of information. Ancient environmental DNA in lake sediments (*sedaDNA*) is one of the latest emerging methods in an ever-growing paleo-limnological toolkit where new proxies emerge as new and improved methods evolve. Yet, this method still needs to emerge from its potential.

I will present results from two studies where we used shotgun metagenomic sequences from lake sediments to investigate environmental changes. The first is a unique sedimentary archive from the Botanical Garden Lake in the center of Copenhagen City. Analysis on *sedaDNA*, and micro- and macrofossils provided us with a detailed insight into the management of the Copenhagen Moat, the fields in the hinterland and the construction of the Botanical Garden. Detailed historical descriptions of introduced plant species inside the Botanical Garden allowed us to benchmark *sedaDNA* identified plant taxa against these historic sources together with the rich macrofossil record. We found that *sedaDNA* was 5 times more sensitive to detect the presence of plant taxa compared to plant macrofossils. However, the amount of DNA reads was not found to reflect the macrofossil counts as there was a strong on/off signal of detection. In addition, we found genetic traces from the third cholera pandemic which had a major outbreak in Copenhagen in 1853. The type strain was identified to be the O-type corresponding to the 1849 Philadelphia specimen from the same pandemic. With additional measurements on

combustion particles (Poly-Aromatic Hydrocarbons), lignin, and Hg concentrations we found significant increases at the onset of industrialization. Even political clean-air initiatives could be identified together with pre-industrial peaks depicting the city's history of large fires and bombardments. We effectively show that lake sediments are a rich source for environmental changes even for depicting the historical record.

In the second study we investigated the biological timing and variability inside the proposed North American 'ice-free' corridor. During the millennia surrounding the Last Glacial Maximum (LGM) (21 thousand years ago [cal kya]) most of Northern 'North America' (Canada) was covered by the Cordilleran and Laurentide ice sheets, effectively cutting off Beringia (northeast Siberia and northwest North America) from ice free parts of North America. Recent geological evidence suggests that this passage was physically open ~15-14 cal kya. However, it is not known when plants and animals colonized the newly deglaciated landscape, and thus when this passage became a biologically viable route for migrating peoples. We used shotgun metagenomic *sedDNA* analysis, macro- and microfossils from three lakes to illuminate the corridor's first colonizers. The preliminary results suggest that an *Artemisia* (sagebrush) steppe environment established prior to 12.5 cal kya including bison and mammoth (*Mammuthus*). At 11.5 cal kya the steppe was replaced by open forest (parkland) including *Populus*, *Salix* and fauna with moose (*Alces*) and elk (*Cervus*).

### **Long-term changes of alpine landscapes revealed by lake sediment DNA**

Gentile Francesco Ficetola<sup>1</sup>, Wentao Chen<sup>1</sup>, Manon Bajard<sup>2</sup>, Johan Pansu<sup>1</sup>, Charline Giguet-Covex<sup>1</sup>, Ludovic Gielly<sup>1</sup>, Philippe Choler<sup>1</sup>, Frédéric Boyer<sup>1</sup>, Fabien Arnaud<sup>2</sup>, Jérôme Poulénard<sup>2</sup>, Pierre Sabatier<sup>2</sup>, and Pierre Taberlet<sup>1</sup>

<sup>1</sup>Laboratoire d'Ecologie Alpine, Université Grenoble-Alpes; <sup>2</sup>EDYTEM, Université Savoie – Mont Blanc, France

Inferring past biodiversity using DNA from natural archives (sediments, permafrost, ...) is a new approach to reconstruct the environment with high-resolution over millennia. However, these studies are often at the local scale. We used sediment DNA metabarcoding to reconstruct changes of vegetation and farming in multiple alpine lake catchments, and to identify the driving factors, by combining analyses of past assemblages with other paleoenvironmental proxies.

We show that analogous changes occurred during the same period across the Alps, with a first peak of human activities during the Roman period determining major landscape changes, and a second increase during the Middle Age. During the last 6,500 years, the impact of human activities on the trajectories of plant communities was more important than climatic oscillations, and communities did not come back to their initial state when human pressure ceased. Integrating data from multiple sites allows a regional reconstruction of human impact through time, and to identify the complex determinants of environmental changes.

### **Needle in a Haystack: Searching for pathogens in ancient human metagenomic datasets**

Meriam Guellil

MedPlag Research Group, Ancient DNA Laboratory, Centre for Ecological and Evolutionary Synthesis, Dept. of Biosciences, University of Oslo, Norway

Next generation sequencing methods allow us to generate huge amounts of data from a single ancient individual. However, usually only the tiniest amount of said data is endogenous and thus of interest for analysis.

One of the biggest challenges in ancient human metagenomics is separating endogenous microbiomes from environmental contaminants be they past or present. This is especially true when searching for pathogens, which tend to only be represented in very small quantities in each and every dataset, if they can be detected at all. Increasingly, the detection of pathogens such as *Yersinia pestis* is done via shotgun sequencing and brings its very own

new set of challenges. This talk will discuss the main challenges involved and how to address them.

**When lipid biomarkers meet gene specific quantification: insight into a 150-year record of phytoplankton community succession controlled by hydroclimatic variability in a tropical lake**

Nolwenn Callac<sup>1</sup>, Yamoah K. K. Afrifa<sup>1</sup>, Ernest Chi Fru<sup>1</sup>, Alan Wiech<sup>1</sup>, Barbara Wohlfarth<sup>1</sup>, Akkaneewut Chabangborn<sup>2</sup> and Rienk H. Smittenberg<sup>1</sup>

<sup>1</sup>*Department of Geological Sciences and Bolin Centre for Climate Research, Stockholm University, 10691 Stockholm, Sweden;* <sup>2</sup>*Departments of Geology, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand*

Climate and human-induced environmental change promotes biological regime shifts, leading to alternate stable states with implications for ecosystem resilience, function and activity. While this has been shown for recent microbial communities, the long-term response of these microbial communities has not been investigated in detail. A sediment gravity core, collected in Lake Nong Thale Prong in southern Thailand, allowed investigating variations in phytoplankton communities by using lipid biomarker analysis and specific gene quantification (quantitative qPCR). The data set shows that the lake was oligotrophic between ~1857-1916 Common Era (CE) and dominated by the green algae *Botryococcus braunii*, which produced characteristic botryococcene lipids. A change to higher silica (Si) input ~1916 CE, related to increased rainfall, concurs with an abrupt takeover by diatom blooms lasting for 50 years. Since the 1970s, a drier and more stable eutrophic state flourished, apparently linked to increased anthropogenic phosphate (P) introduction. This eutrophic state led to primary productivity predominated by Cyanobacteria and *Botryococcus sp.*, that do not produce botryococcene lipids. Our results indicate that a combined qPCR and lipid biomarker approach provide an efficient method for tracking centennial-scale hydroclimatic and anthropogenic feedback processes in tropical lake ecosystems.